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Negative Chemical Ionization Studies of Human and Food Chain Contamination with Xenobiotic Chemicals

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16. ABSTRACT

Negative chemical ionization mass spectrometry with a mixture of isobutane, methylene chloride, and oxygen as the reagent gas has been used to explore contamination of environmental substrates with xenobiotic chemicals. The substrates in question, fish tissue, human seminal plasma, and human adipose tissue, were cleaned up by one of the following three cleanup procedures: (1): continuous liquid-liquid extraction steam distillation; (2) gel-permeation chromatography; and (3) adsorption on activated carbon followed by elution with toluene. The third procedure was used only for the examination of planar polychlorinated aromatic hydrocarbons in environmental samples. Using these techniques, we have found evidence for contamination of fish samples with polychloronaphthalenes, polychlorostyrenes, polychlorobiphenyls, polychlorodibenzofurans, and polychlorodibenzodioxins among other chemicals. The polychlorodibenzodioxins appeared only in the spectra of extracts of fish obtained from the Tittabawassee River at Midland, Michigan. The polychlorodibenzofuran ions appeared in NCI mass spectra of fish taht were significantly contaminated (above 2 ppm) with polychlorobiphenyls. Toxic substances occurring in human seminal plasma included pentachlorophenol, hexachlorobenzene, DDT metabolites, and polychlorobiphenyls. We have investigated toxic substances in human seminal plasma because of the apparent decrease in sperm density in U.S. males over the last 30 years.

Results of screening human adipose tissue for contamination with xenobiotic chemicals have been largely coincident with results of the EPA human monitoring program. Polychlorobiphenyls, DDT metabolites, nonachlor, and chlordane have appeared in most samples examined. Detection limits for all of these chemicals were of the order of 1 ppb.

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Negative Chemical Ionization Studies of Human and Food Chain Contamination with Xenobiotic Chemicals

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Negative chemical ionization mass spectrometry with a mixture of isobutane, methylene chloride, and oxygen as the reagent gas has been used to explore contamination of environmental substrates with xenobiotic chemicals. The substrates in question, fish tissue, human seminal plasma, and human adipose tissue, were cleaned up by one of the following three cleanup procedures: (1) continuous liquid-liquid extraction steam distillation; (2) gel-permeation chromatography; and (3) adsorption on activated carbon followed by elution with toluene. The third procedure was used only for the examination of planar polychlorinated aromatic hydrocarbons in environmental samples. Using these techniques, we have found evidence for contamination of fish samples with polychloronaphthalenes, polychlorostyrenes, polychlorobiphenyls, polychlorodibenzofurans, and polychlorodibenzodioxins among other chemicals. The polychlorodibenzodioxins appeared only in the spectra of extracts of fish obtained from the Tittabawassee River at Midland Michigan. The polychlorodibenzofuran ions appeared in NCI mass spectra of fish that were significantly contaminated (above 2 ppm) with polychlorobiphenyls. Toxic substances occurring in human seminal plasma included pentachlorophenol, hexachlorobenzene, DDT metabolites, and polychlorobiphenyls. We have investigated toxic substances in human seminal plasma because of the apparent decrease in sperm density in U.S. males over the last 30 years.

Results of screening human adipose tissue for contamination with xenobiotic chemicals have been largely coincident with results of the EPA human monitoring program. Polychlorobiphenyls, DDT metabolites, nonachlor, and chlordane have appeared in most samples examined. Detection limits for all of these chemicals were of the order of 1 ppb.

Introduction

Xenobiotic chemicals are chemicals that are foreign to biology. In common usage, the term xenobiotic also connotes a hazard to biology. Man-made toxic substances, xenobiotic chemicals, span the range from analogs of alkaloidal poisons to highly toxic industrial contaminants such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and disulfur decafluoride. Analytical methodologies that are designed to detect residue concentrations of man-made toxic substances in the environment should ideally be selective for those chemicals in the presence of biomolecules. The unique feature of toxic substances, with the exception of alkaloidal analogs, that can be used to advantage in designing schemes for their detection is the fact that virtually all of these molecules are either oxidizing agents or alkylating agents or both. Oxidizing agents or alkylating agents will capture ei-

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ther thermal electrons or thermalized gas-phase nucleophiles to form anionic complexes in the gas phase. A considerable selectivity in detection arises from the fact that biomolecules, in contrast to toxic substances, do not in general attach thermal electrons or gas phase nucleophiles. This feature of biomolecules is simply a result of the fact that biologicals are highly reduced and have in general a very large number of high-energy electrons. Electron affinities for biomolecules are generally negative, in contrast to those of commonly occurring toxic substances and proximal carcinogenic agents. There are exceptions to the generalization noted above; these include free fatty acids, which attach gas phase nucleophiles very readily, and molecules from the electron transport chain such as the cytochromes which will readily attach thermal electrons. It is generally not difficult to separate carboxylic acids or molecules from the electron transport chain from residues of xenobiotic chemicals that one wishes to investigate.

Before embarking on a study of toxic residues in human samples and items from the food chain, it is necessary to come to grips with the question of "levels of concern". A "zero" tolerance limit for carcinogenic substances in the food chain or humans is a completely unscientific statement because this statement is neither qualified by the sensitivity of the analytical methodology available or the toxicological effect of the substance at any stated level. The generally accepted dictum that "there is no threshold dose for a carcinogen" must be tempered with the knowledge that there is a level below which the dose is insignificant when compared to the background dose; i.e. cosmic radiation, natural radioactivity, and ubiquitous fungal toxins. Aflatoxin-B, a molecule which is virtually as toxic as TCDD, exists in selected items in the food chain at levels approaching 10 ppb.

One analysis that may shed light on the question of "levels of concern" is the number of molecules per cell that one can find in a bio-sample at different levels of contamination. For a molecule with a molecular weight of 300 daltons a concentration of 1 ppt in a mammalian tissue sample amounts to approximately two molecules per cell (*1*). It is our contention that with the exception of exceptionally toxic molecules, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), these levels in human cells are below the level of concern. Part-per-trillion levels in environmental samples from either the food chain or the water supply may be of concern in view of the fact that bioconcentration factors for many substances, e.g., octachlorostyrene, are of the order of 10^6 (*2*).

With the considerations above we have decided to set the level for our toxicological screening of human samples at 1 ppb. This level corresponds to roughly 1000 molecules per cell and for molecules with significant toxicity it is definitely a "level of concern". For items from the food chain and the water supply, lower levels may be important, particularly in attempts to define the origins of the human contamination that becomes evident in a screening program.

In order to take advantage of the differential sensitivity to anion-forming reactions that exist between xenobiotic molecules and the molecules of biology, we have been employing negative chemical ionization (NCI) mass spectrometry as the primary analytical tool in our screening program. Depending upon the analytical objective, we employ one of two different reagent gas mixtures, namely: isobutane with traces of oxygen (*3*) or methylene chloride with traces of oxygen (*4*). The dominant ionization processes that occur in both of these gases are listed in Eqs. (1)-(5).

Resonance capture:

$$M + e_s \longrightarrow M^{\pm *} \rightarrow M^{\pm} \qquad (1)$$

Dissociative capture:

$$M - X + e_s \longrightarrow M- + X^{\cdot} \qquad (2)$$

Anion association:

$$M + X^- \rightarrow MX^- \qquad (3)$$

Deprotonation:

$$AH + B^- \rightarrow A^- + BH \qquad (4)$$

Oxygen exchange:

$$A{-}Y^{\cdot -} + O_2 \rightarrow AO^- + YO^{\cdot} \qquad (5)$$

Methylene chloride appears to give the best results for screening for aliphatic polyhalogen compounds such as are found in technical grade chlordane. Hydrocarbon-oxygen mixtures appear to have higher sensitivity for detection of aromatic polyhalides and polynuclear aromatic hydrocarbons. Since we have been concerned with food chain contamination with aromatic polyhalides such as TCDD, most of the work described below has been done with isobutane-oxygen mixtures. Detection limits using these conditions are ca. 1 ng; i.e., 1 ppb for a 1 g sample.

Sample cleanup, the separation of the molecules of interest from the matrix in which they are

found, is a crucial part of any screening effort. Obviously, the fewer the number of steps in the cleanup procedure, the more likely acceptable recoveries will be obtained and the less likely contamination of the sample will occur. The screening efforts reported here have used either continuous extraction or steam distillation (*5*) for initial extraction. Gel-permeation chromatography (*6*) or adsorption chromatography with activated carbon (*7*) have been used as subsequent cleanup steps. We have attempted to avoid the use of adsorption chromatography because of generally low recoveries of polar xenobiotic molecules such as pentachlorophenol. Activated carbon-coated polyurethane adsorption chromatography was used for the highly selective isolation of planar aromatic hydrocarbons such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (*7*) where interferences such as polychlorinated biphenyls can severely complicate the analytical procedure (*8*).

Experimental

Mass Spectrometry

Negative chemical ionization mass spectra were obtained by direct probe introduction with the use of an AEI MS-902 mass spectrometer equipped with an SRIC chemical ionization source. Ionization was initiated with 470-V electrons with a regulated emission current of 0.25 mA. Source pressures were monitered by use of an MKS Baratron which was operated at the source potential (−8 kV) and isolated from earth. Spectral-quality methylene chloride was distilled prior to use; the isobutane used in these experiments was Matheson Research grade.

Steam Distillation Cleanup

The steam distillation head used for sample cleanup is very similar to that described by Veith

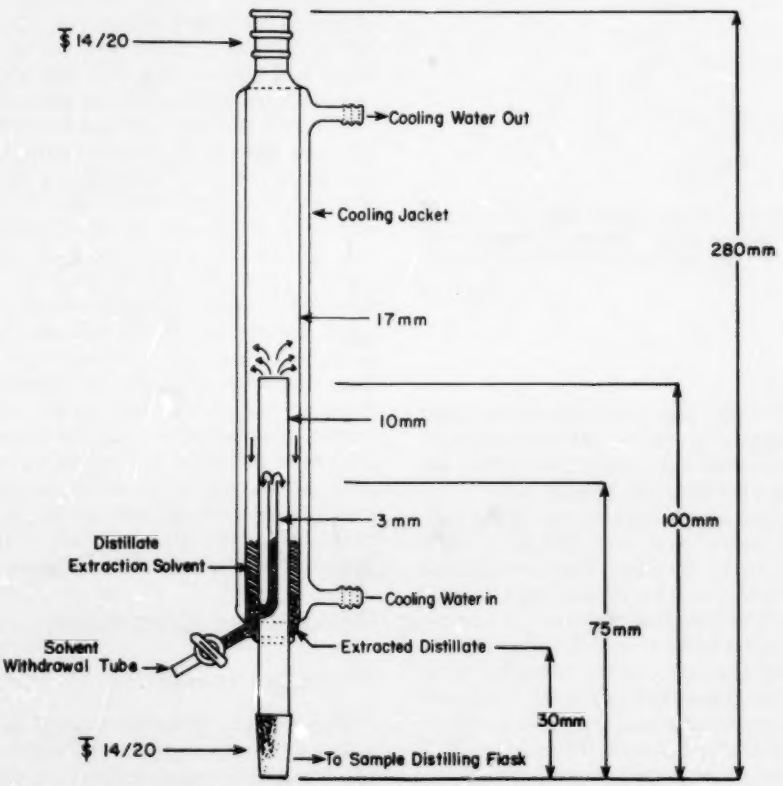

FIGURE 1. Steam distillation cleanup head for micro sample cleanup.

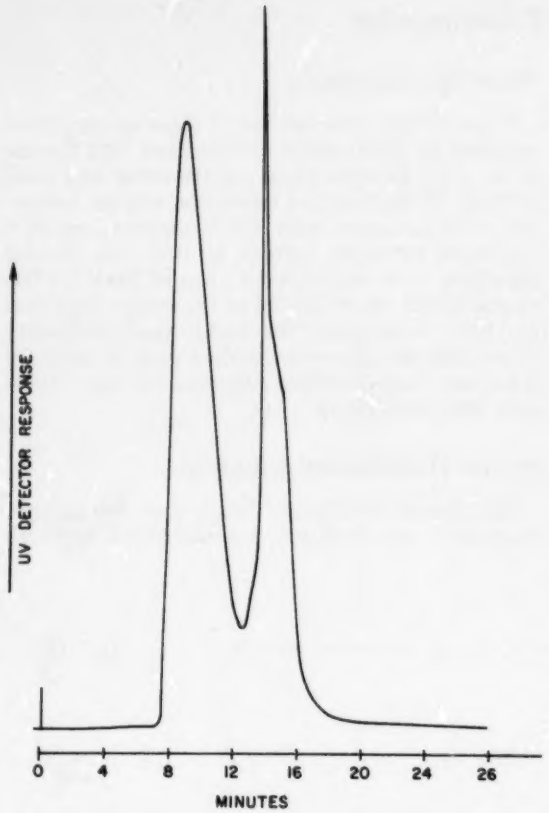

FIGURE 2. Gel permeation chromatographic trace of an extract of 50 mg of Lake Ontario trout. Column: Bio-Beads SX-8, 500 mm × 10 mm; solvent: methylene chloride/cyclohexane, 1:1, 2 ml/min.

and Kiwus (*5*). The dimensions of the steam head were modified slightly to improve sample recoveries. The dimensions of the steam head that we presently use are illustrated in Figure 1.

In a typical experiment, 250 mg of fresh adipose tissue were macerated with 100 ml of 10% sulfuric acid in a Vertis blender. The homogenate was warmed at 80°C for 40 min and then steam-distilled into 2 ml of distilled-in-glass 2,2,4-trimethylpentane. After 1 hr of steam distillation, the aqueous and isooctane layers were removed from the steam head and separated by use of a Pasteur pipet. Samples were concentrated by using a Snyder column and either examined directly by NCI mass spectrometry or subjected to gel-permeation chromatography prior to analysis.

Gel-Permeation Chromatographic Cleanup

A fish sample (150 mg) was mixed thoroughly with 2.0 g anhydrous sodium sulfate and Soxhlet-extracted with hexane/acetone (1:1) for 4 hr. The resulting oil was concentrated to 1.0 ml for GPC. The GPC system is similar to the system described by Kuehl and Leonard (*6*) by using a 1.0 cm × 50.0 cm column of Bio-Rad SX-8 at a flow rate of 2.0 ml/min cyclohexane/CH_2Cl_2. A Varian UV detector (254 nm) was used to monitor the column effluent.

Cleanup Procedure for Planar Aromatic Hydrocarbons

A fish sample (25 g) was mixed thoroughly with 100 g of anhydrous sodium sulfate, packed in a glass column and extracted with 250 ml of methylene chloride. The solvent was removed by rotary evaporation and the extract subjected to gel-permeation chromatography (GPC). Extract, 1 g or less, was chromatographed on 60 g of Bio-Beads S-X3 in a 2.5 × 48 cm column by using a mixture of cyclohexane and methylene chloride (1:1). The 160 to 220 ml fraction was collected, as previous work had shown that this fraction contained all contaminant residues of interest (*6*).

The fraction from gel permeation chromatography was reduced to 30 ml and applied to a column containing 1 g of shredded polyurethane foam which had been coated with 5% AMOCO PX-21 carbon (<44 μm particle size) packed in a 1 × 8.5 cm column. The following elution solvent sequence was used: 30 ml ethyl acetate, 20 ml 4% benzene in ethyl acetate, 20 ml 20% benzene in ethyl acetate, 20 ml 50% benzene in ethyl acetate. At this point the column was washed in a reverse flow direction with 30 ml toluene. The toluene fraction containing the planar aromatic hydrocarbon residues was carefully evaporated under a stream of nitrogen in a warm water bath to a volume of 50 μl and analyzed by either electron capture gas chromatography or negative chemical ionization mass spectrometry with isobutane/oxygen being used as the reagent gas.

Results and Discussion

Cleanup Procedures

The cleanup procedures used in this study are both very simple and quite selective for separating xenobiotic chemicals from tissue and other environmental matrices. In previous studies (*9*)

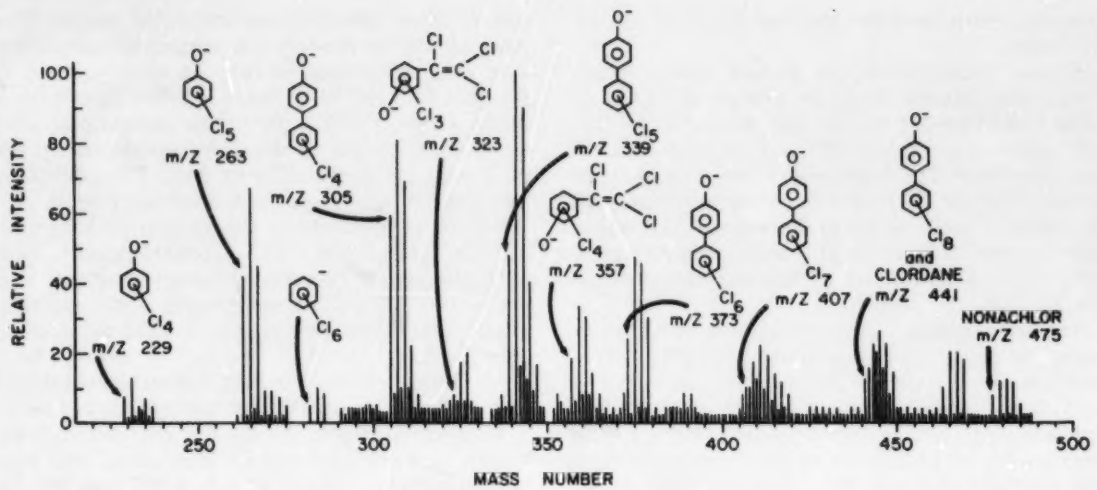

FIGURE 3. Negative chemical ionization mass spectrum of a steam distillate of 150 mg of Lake Ontario trout. Source temperature, 150°C; pressure, 0.45 torr; reagent gas, isobutane oxygen, 10:1.

we have shown that sample recoveries for two- to four-ring polynuclear aromatic hydrocarbons with a steam distillation cleanup have ranged from 95 to 105%. A disadvantage of steam distillation as a cleanup procedure for tissue samples when using negative chemical ionization mass spectrometry as a final detector is the codistillation of free fatty acids. If the free fatty acid concentration in the steam distillate exceeds a critical value, the NCI spectrum of the distillate will be dominated by ions from the carboxylic acids. This results in a decrease in sensitivity for xenobiotic chemicals. In cases where direct examination of the steam distillate was not possible because of inter-

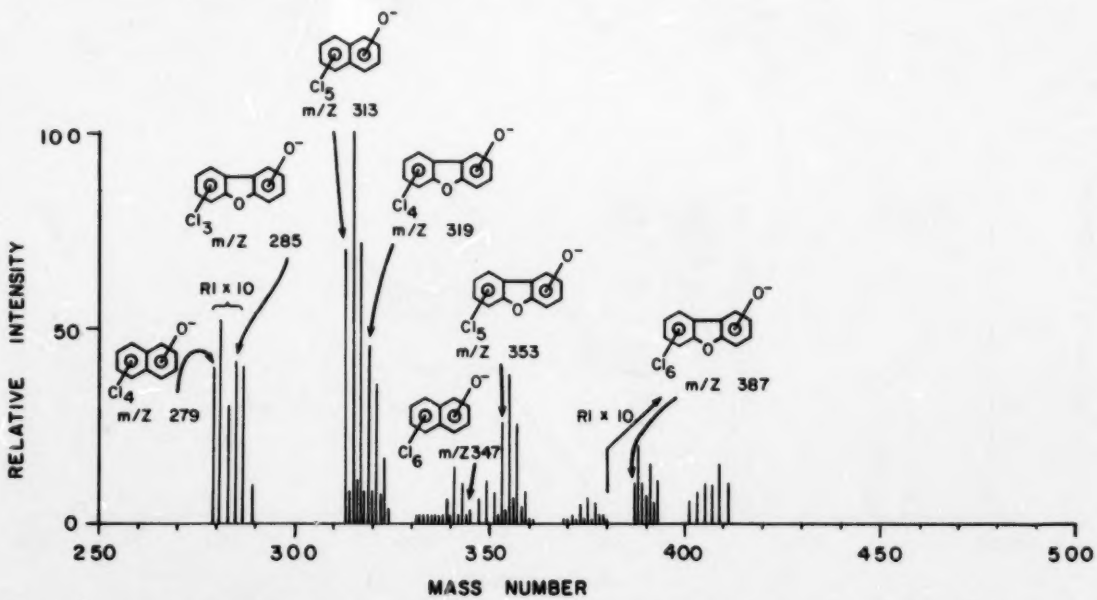

FIGURE 4. Negative chemical ionization mass spectrum of planar molecules in Ohio River Carp, Marietta, Ohio, 1977. Source temperature, 150°C; pressure, 0.46 torr; reagent gas, isobutane/oxygen, 10:1; cleanup on activated carbon.

ferences, samples were chromatographed on the GPC unit.

Figure 2 illustrates the gel-permeation chromatogram obtained from an extract of 50 mg of Lake Ontario trout tissue. The resolution in this GPC trace is typical of GPC separations that we have observed. The fraction that appears at large elution volumes (26 to 36 ml) contains virtually all the xenobiotic chemicals in the sample. The isobutane/oxygen NCI mass spectrum obtained on a GPC cleaned-up extract of 150 mg of the same trout appears in Figure 3.

The combination of gel-permeation chromatography followed by carbon-foam adsorption chromatography provides a cleanup procedure that is exceptionally selective for planar aromatic hydrocarbons and their polychlorinated analogs. The elimination of nonplanar molecule interferences substantially enhances the sensitivity for detection of small amounts of planar polyhalogenated polynuclear aromatic hydrocarbons.

Negative Chemical Ionization Screening, Fish Samples

The NCI mass spectrum of an extract of Lake Ontario trout which appears in Figure 3 is fairly typical of the spectra that one obtains from samples of large fish from contaminated waters. The ratio of the ion clusters for pentachlorophenoxide and tetrachlorophenoxide in this sample is roughly 5.8 ($m/z = 263$:$m/z = 229$). This ratio is about a factor of 2 higher than the ratio of pentachlorophenoxide to tetrachlorophenoxide in the NCI mass spectrum of Dowcide G. The additional ion current due to pentachlorophenoxide in this spectrum is the result of the oxygen exchange reaction [reaction (5)] occurring on hexachlorobenzene. Oxygen exchange reaction ions occur for the polyhalobiphenyls (*10*), starting with tetrachlorobiphenyl at $m/z = 271$ followed by pentachlorobiphenyl ($m/z = 305$), hexachlorobiphenyl ($m/z = 339$), heptachlorobiphenyl ($m/z = 407$) and octachlorobiphenyl ($m/z = 441$).

Ions corresponding to the oxygen exchange reaction occurring on octachlorostyrene and heptachlorostyrene appear at $m/z = 357$ and 323, respectively, in agreement with spectra of standards. The other major ion currents occurring in this spectrum correspond to chloride attachment [reaction (3)] ions of chlordane and nonachlor at $m/z = 441$ and 475, respectively, and an unknown 7-chlorine cluster that appears at $m/z = 463$. The presence of abundant quantities of polyhalobiphenyls and polyhalophenols would virtually obscure the presence of lower levels of planar

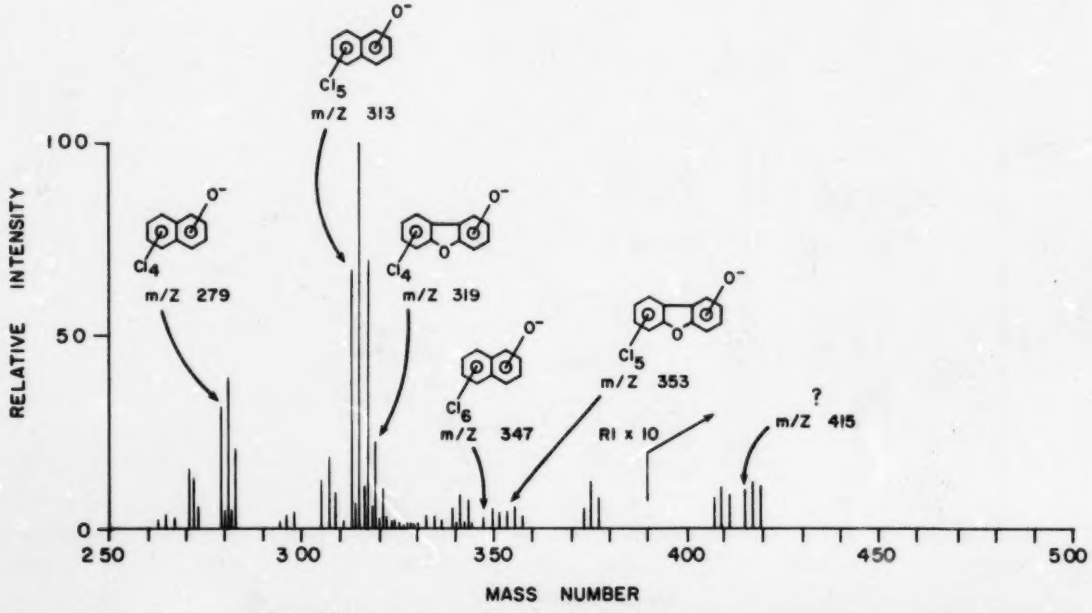

FIGURE 5. Negative chemical ionization mass spectrum of planar molecules from Connecticut River perch, Wilson, Connecticut, 1970. Source conditions and cleanup identical to those in Figure 4.

polychlorinated polynuclear aromatics such as the dibenzofurans and dioxins.

In order specifically to examine fish samples for the presence of polychlorinated dibenzofurans and polychlorinated dibenzodioxins, we subjected a series of samples to the GPC/activated carbon cleanup procedure. Adsorption on activated carbon followed by desorption with toluene is an extremely selective procedure for concentrating planar polychloroaromatic hydrocarbons. Figure 4 presents the NCI mass spectrum obtained from an extract of carp that was caught in the Ohio River at Marietta in 1971. The carp sample showed a 5.7 ppm concentration of polychlorobiphenyls as determined by electron capture gas chromatography. The NCI mass spectrum obtained with isobutane/oxygen shows a series of oxygen exchange ions [reaction (5)] with masses and isotopic abundances that correspond to those ions one would expect from a series of polychloronaphthalenes and polychlorodibenzofurans (*11*). The oxygen exchange ions for the polychloronaphthalenes appear as a series of ions at m/z = 279, 313, and 347, corresponding to the oxygen exchange ions from pentachloronaphthalene, hexachloronaphthalene, and heptachloronaphthalene, respectively. The oxygen exchange reaction ions for the polychlorodibenzofurans appear as a series of ions at m/z = 255, 319, 353, and 387, corresponding to tetrachlorodibenzofuran, pentachlorodibenzofuran, hexachlorodibenzofuran, and heptachlorodibenzofuran, respectively.

Figure 5 illustrates the NCI mass spectrum of a GPC/activated carbon cleaned-up extract of a perch that was obtained from the Connecticut River at Wilson, Connecticut in 1970. This fish contained 2.4 ppm of polychlorobiphenyls as evidenced by electron capture gas chromatography. The abundance of ions corresponding to polychlorodibenzofurans is significantly lower in this fish than in the previous example. The spectrum in Figure 5 is dominated by the oxygen exchange ions from the polyhalonaphthalenes at m/z = 297, 313, and 347. The concentrations of the polychlorodibenzofurans in this sample with reference to the polyhalonaphthalenes must have been roughly one-half that shown in the spectrum in Figure 4.

Figures 6 and 7 show the NCI mass spectra of the planar polychloropolynuclear aromatic hydrocarbons from composite fish samples obtained from the Tittabawassee River near Midland, Michigan. The composites in both cases were made up of samples of large and small carp and bass. The spectrum in Figure 6 is that obtained

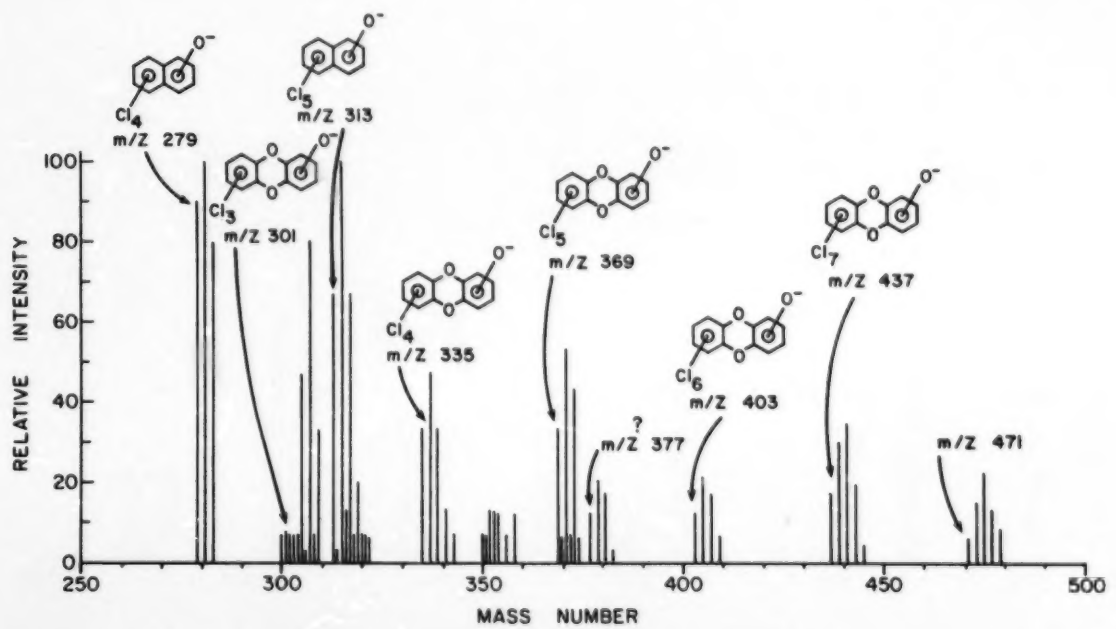

FIGURE 6. Negative chemical ionization mass spectrum of planar molecules in composite samples of Tittabawassee River carp, Emerson Park, Midland, Michigan, 1977. Source conditions and cleanup equivalent to those in Figure 4.

from the extracts of fish obtained above the Dow Dam at Emerson Park in Midland, Michigan in 1977. The spectrum in Figure 7 is that obtained from fish below the Dow Dam where the fish would have access to water from the waste chemical outfalls from Dow Chemical, Inc. The oxygen exchange reaction ion series that are characteristic of the polychlorodibenzodioxins (*12,13*) appear in the spectra. The spectra illustrated in Figures 6 and 7 are the only spectra that we have obtained on extracts of animals tissues from natural environments that have given evidence for the presence of part-per-billion or higher concentrations of polychlorodibenzodioxins. The abundance of the oxygen exchange ions for the polychlorodibenzodioxins in the fish obtained below the Dow Dam is higher with reference to the polychloronaphthalenes in the sample than the corresponding ion abundances in the fish samples obtained at Emerson Park. This would be consistent with a contribution to the dioxin burden in that area from the Dow Chemical waste water outfall. The presence of ions corresponding to the polychlorodibenzodioxins in fish obtained above the Dow Dam strongly suggests atmospheric transport as one of the routes for distribution of dioxins in that locale. A combined sample of fish from above and below the Dow Dam was examined by GC-EI mass spectrometry using a Finnegan 4023 GC-mass spectrometer. One peak in the GC mass spectrum had both a GC retention time and mass spectrum that was coincident with that obtained from a standard sample of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The ion current at $m/z = 301$ in Figures 6 and 7 correspond to the sum of the isomeric tetrachlorodibenzodioxins in these samples.

Our findings of planar polychlorinated polynuclear aromatic hydrocarbons in fish samples are summarized in Table 1. A positive finding is that NCI screening revealed the presence of an ion with mass and isotopic abundance corresponding to that of the oxygen exchange reaction ion for the indicated chemical structures. The occurrences of the polyhalodibenzofurans are very likely related to the occurrence of high levels of polychlorobiphenyls in the aquasphere at the indicated sampling locations. Since the polychlorodibenzodioxins appeared only in samples obtained in the Tittabawassee River, it seems relatively safe to assume that simple combustion processes are not responsible for the origin of these compounds in the environment.

Numerous recent reports have signaled the environmental hazards associated with combustion of polyhalogenated organics, particularly poly-

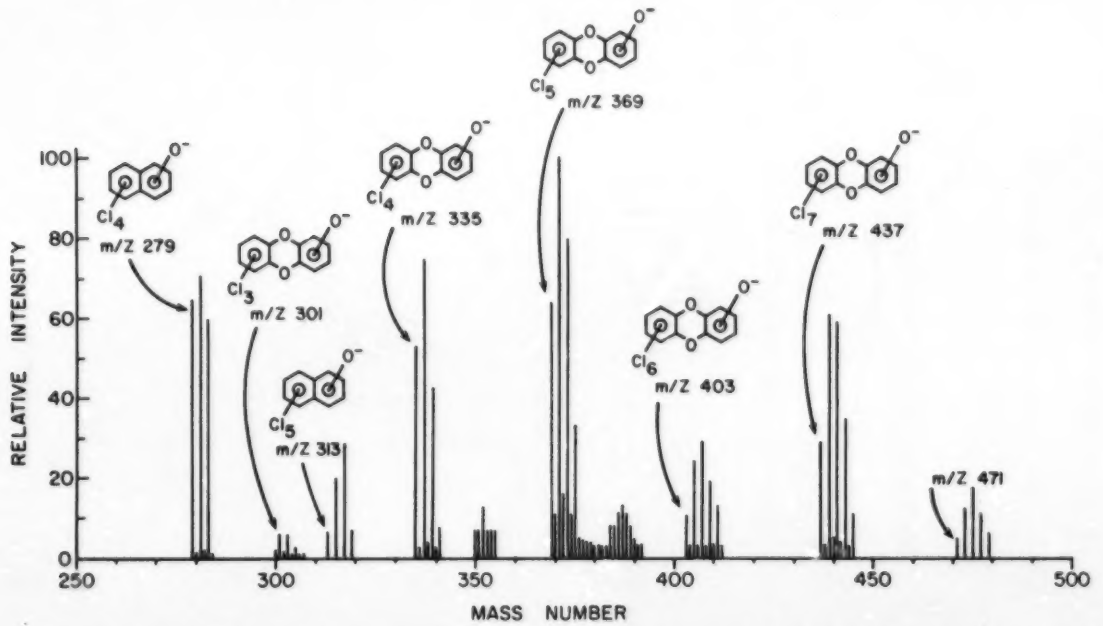

FIGURE 7. Negative chemical ionization mass spectrum of planar molecules in a composite sample of Tittabawassee River Carp collected below the Dow Dam in Midland Michigan, 1977. Source conditions and cleanup equivalent to those in Figure 4.

Table 1. Polychorodibenzolioxins, dibenzofurans, and naphthalenes occurring in fish collected in the midwestern and northeastern United States.[a]

Sample	Polychlorodibenzodioxins					Polydibenzofurans					Naphthalenes				
	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl
Ohio R., Cincinnati, 1970	0	0	0	0	0	+	+	+	0	0	+	+	+	+	0
Ohio R., Marietta, 1971	0	0	0	0	0	+	+	+	+	0	+	+	+	+	0
Ohio R., Cincinnati, 1974	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0
Lake Michigan, Saug., 1974	0	0	0	0	0	+	+	+	0	0	+	+	+	+	0
Ohio R., Marietta, 1970	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0
Connecticut R., Wilson, 1974	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0
Connecticut R., Wilson, 1970	0	0	0	0	0	+	+	+	0	0	+	+	+	+	0
Hudson R., Wappinger Falls, 1974	0	0	0	0	0	0	0	+	0	0	+	+	+	0	0
Ohio R., Marietta, 1974	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0
Lake Michigan, Shebdygan, 1974	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0
Hudson R, Wapinger Falls, 1976	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0
Tittabawassee R., Dow Dam, 1977	+	+	+	+	+	0	0	0	0	0	0	+	+	0	0
Tittabawassee R., Emerson Park, 1977	+	+	+	+	+	0	0	0	0	0	0	+	+	0	0

[a] Plus (+) denotes ion found.

Table 2. Male fertility potential in the United States.

Investigators	Date	No. of cases	Sperm density/ml
Macomber and Sanders (*21*)	1929	271	Avg 100M ~50% > 100M
Hotchkiss et al. (*22*)	1938	200[a]	Avg. 120M 0.5% < 20M 25% < 60M
Farris (*23*)	1949	49	Avg. 145M Range 50–321M
Falk and Kaufman (*24*)	1950	100	Avg. 100.7M
MacLeod and Gold (*25*)	1951	1000	Avg. 107M 29% < 60M 44% > 100M
Nelson and Bunge (*26*)	1974	386	Avg. 48M
Rehan, Sobrero, and Fertig (*27*)	1975	1300	Avg. 79M Median 65M
Sobrero and Rehan (*28*)	1975	100[a]	Avg. 81M Median 68M
Wharton (*29*)	1978	260	Median 80M

[a] Wives pregnant at the time

chlorophenols (*14–17*). Public discussion has suggested that the polychlorodibenzodioxins that appear in the environment may have their origins in the combustion of organochlorine compounds (*18*). While the environmental hazard associated with combustion of organochlorine compounds is clearly real (*14–17*), it seems unlikely that simple combustion was responsible for the unique occurrence of polychlorodibenzodioxins in fish from the Tittabawassee River.

The very general occurrence of polyhalonaphthalenes in the fish samples indicates a more wide-spread contamination of the biosphere with these compounds than we had previously suspected. Polyhalonaphthalenes have physical and chemical properties that are very closely related to polychlorobiphenyls and they have the same uses.

NCI Screening of Human Seminal Fluid

There is evidence in the literature that male fertility potential in the United States has decreased significantly in the period since 1951. Table 2 summarizes the results of a number of investigations (*19–27*) of male fertility potential in the United States in the period from 1929 to 1978. It is clear from the data presented in Table 2 that the average sperm density for human males in the United States in the period between 1929 and

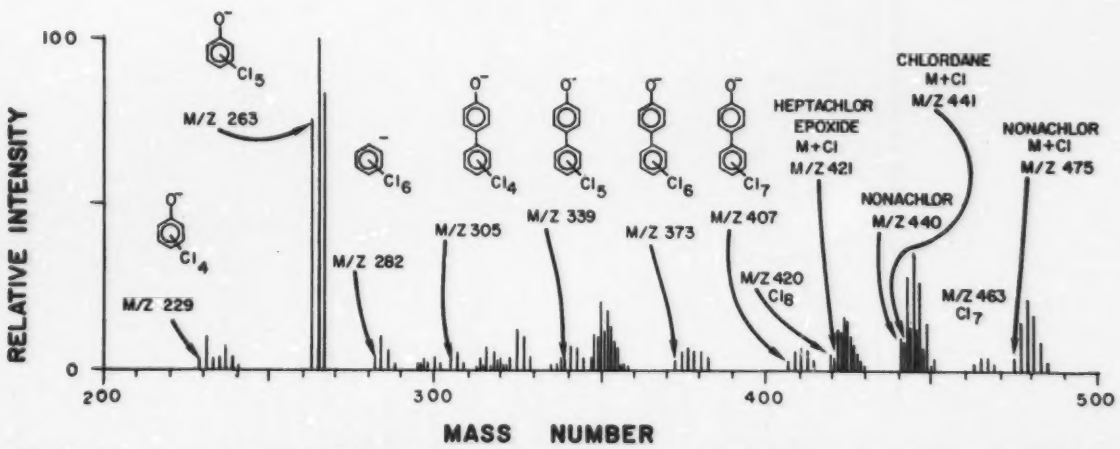

FIGURE 8. Electron capture gas chromatographic trace of a steam distillate of 2 ml of human seminal fluid and a procedural blank. On 3% OV-101, isothermal, 190°D.

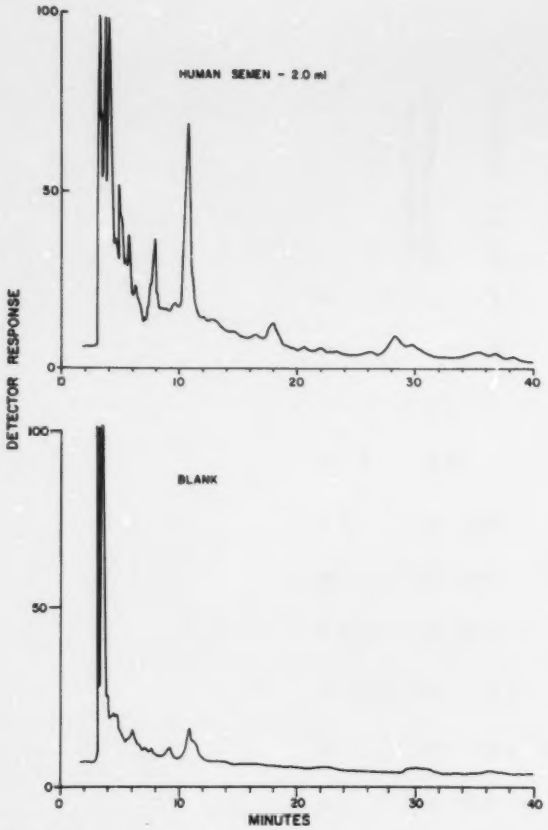

FIGURE 9. Negative chemical ionization mass spectrum of a steam distillate of human seminal fluid. Source conditions equivalent to those in Figure 4.

1951 ranged from 100 to 145M/ml. Reports subsequent to 1970 have indicated a range of average sperm densities for United States males between 48 and 80M/ml. There are a number of ways of reporting sperm densities; logarithmic scales have been suggested, and median values have been used in the place of mean to offset the effect of a small number of samples with high sperm density on the appearance of the density distribution. Regardless of the means of presentation, the data in Table I strongly suggest that there has been a significant decrease in sperm density in United States males in the last 30 years. A number of factors suggested as potential causes for this decrease are increased use of tight-fitting underpants, increased frequency of ejaculation, and increased exposure to toxic substances.

The reason for believing that a study of sperm density and toxic substances in humans could provide useful information concerning environmental health problems are straight-forward and based on the biology of spermatogenesis. The formation of sperm in the seminiferous tubules requires a sequence of eight cell divisions. There are six normal mitotic steps followed by two meiotic steps which are required for the production of each sperm. Inhibitors of cell division will thus have a n^8 effect on sperm density, where n is a number less than 1. There should be, by this mechanism, a substantial magnification in the effect of inhibitors of cell division when one examines sperm density as compared to other biological parameters. Inhibitors of cell division are very generally mutagenic agents with the exception of certain alkaloidal poisons. Most of the well known carcinogenic substances will inhibit cell division.

Wyrobek has shown that sperm density decreased approximately linearly (*28*) and the number of abnormal sperm cells increased approximately linearly (*29*) with the number of tobacco cigarettes smoked per day. In the same study, mutagenic agents were shown to increase significantly the number of abnormal sperm and decrease sperm densities in mice. The existence of the testicular membrane barrier, which is analogous to the blood-brain barrier, makes it difficult to interpret cases in which mutagens do not have a significant impact on either sperm density or sperm abnormalities.

Our approach to the study of toxic substances and sperm density is analytic. We hope to obtain a sufficient body of data using NCI screening to be able to make meaningful correlations between the appearance of specific toxic substances and low sperm densities. The cleanup procedure that we have used is steam distillation from an acidified sample. Figure 8 presents the electron capture gas-chromatogram from a steam distilled sample of human seminal fluid and a procedural blank. The large peak with a retention time of approximately 11 min. corresponds to *p,p'*-DDE.

Figure 9 illustrates an NCI mass spectrum (isobutane/oxygen) of a similar seminal fluid extract. It is interesting to note that DDE does not appear in this spectrum, although it certainly existed in the sample; however, the *p,p'*-DDMU was identified at much lower levels. The differential sensitivity for DDE as compared to DDMU is very likely due to the higher electrical symmetry of DDE. The spectrum in Figure 9 illustrates the presence of polychlorophenols and polychlorobiphenyls in this sample, along with a number of unidentified ions. The Cl_5 ion cluster at m/z = 323 appeared fairly frequently in our study of human urines (*30*). This ion may be the chloride

Table 3. Dot matrix of occurrences of polyhalogenated ions in negative chemical ionization mass spectral screening of human seminal fluid.

		Sample (sperm/ml × 10^3)																
m/z	No. Cl	(8)	(11)	(12)	(19)	(20)	(20)	(20)	(29)	(106)	(120)	(146)	(198)	(227)	(229)	(—)[a]	(—)[a]	(—)[a]	Compound identification
212	3	●	●	●	—	—	●	●	—	●	●	●	●	●	●	—	—	—	
229	4	—	—	●	●	—	●	●	—	—	—	—	●	●	—	—	—	—	Tetrachlorophenol
231	4	●	—	●		●	●	●	—	●	●	●	●	●	●	—	—	—	
263	5	●	—	●	●	●	●	●	—	●	●	●	●	●	●	—	—	—	Pentachlorophenol
285	3	—	—	—	●	—	—	—	—	—	—	—	—	—	—	—	—	—	
287	5	—	—	—	—	—	●	—	—	—	—	—	—	—	—	—	—	—	
301	4	—	—	—	—	—	—	—	—	●	—	—	—	—	—	—	—	—	
305	4	—	●	●	—	—	—	●	●	—	—	●	—	●	—	—	—	—	Pentachlorobiphenyl
313	5	—	—	●	—	—	—	—	—	—	—	●	—	—	●	—	—	—	
317	4	●	●	●	—	—	●	●	●	●	—	●	●	●	●	—	—	—	*p, p'*-DDMU
321	4	●	—	—	●	—	—	—	—	—	—	—	—	—	—	—	—	—	
323	6	—	—	—	—	—	●	—	—	—	—	—	—	—	—	—	—	—	
339	5	—	●	●	●	—	●	●	●	●	●	●	●	●	—	—	●	●	Hexachlorobiphenyl
343	3	—	●	●	—	—	●	●	●	—	—	●	●	●	—	—	—	—	
345	3	—	—	—	—	—	—	—	—	—	—	—	—	—	●	—	—	—	
373	6	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	—	—	Heptachlorobiphenyl
407	7	—	●	—	—	—	—	—	—	—	●	—	—	—	—	—	—	—	Octachlorobiphenyl
427	7	●	—	—	—	—	—	—	—	—	●	—	—	—	—	—	—	—	
429	5	—	—	—	—	—	—	—	—	—	●	●	—	—	—	—	—	—	
447	5	—	—	—	—	—	—	—	—	—	—	●	—	—	—	—	—	—	
463	7	●	●	●	●	●	●	●	●	●	●	●	●	●	●	—	●	●	

[a] Procedural blanks

Table 4. Dot matrix of occurrences of polyhalogenated ions in negative chemical ionization screening of human adipose tissue.

Mass	No. Cl	20-20-2-MW-I	2-20-1-MW-I	2-24-8-MW-I	2-24-7-MW-I	2-24-5-MW-I	2-24-6-MW-I	2-20-3-MW-I	2-20-4-MW-I	Compound
217	3	●	—	—	—	—	—	—	—	
231	4	●	—	—	—	—	—	—	—	
243	3	●	—	—	—	—	—	—	—	
263	5	●	—	—	—	—	—	●	—	Pentachlorophenol
294	4	—	●	—	—	—	—	●	—	
305	5	●	●	●	●	●	●	●	●	Pentachlorobiphenyl
307	4	—	—	—	—	—	—	●	—	
314	5	—	●	—	—	—	—	—	—	
317	4	●	●	●	●	●	—	●	●	p, p'-DDMU
323	4	—	—	—	—	—	—	●	—	
339	5	●	—	—	—	—	—	●	●	Hexachlorobiphenyl
341	4	—	●	—	●	●	●	●	●	
350	4	—	●	—	—	—	—	—	—	
351	5	—	—	—	—	—	—	●	—	p, p'-DDE
373	6	●	●	●	●	●	●	●	●	Heptachlorobiphenyl
375	5	●	●	—	●	—	●	●	●	
387	6	—	—	—	—	—	—	●	—	p, p'-DDT
407	7	●	●	●	●	●	●	●	●	Octachlorobiphenyl
409	6	—	●	—	●	—	●	●	—	
420	8	—	●	●	●	●	●	●	—	
421	7	—	●	●	●	●	●	●	—	Heptachlor epoxide
440	9	—	●	●	●	●	●	●	—	Nonachlor
441	9	—	●	●	●	●	●	●	●	Chlordane
463	7	●	●	●	●	●	●	●	●	
475	10	—	●	●	●	●	●	●	●	Nonachlor

adduct of a tetrachlorophenoxyacetic acid; however, to our knowledge, that compound is not a commercial product. The ions at $m/z = 447$ and $m/z = 463$ occur frequently, and we are presently actively investigating their structure.

In order to develop statistically significant data on sperm density and toxic substances in seminal fluid it will be necessary to examine a very large number of samples. The general approach that we intend to use in analyzing the data is illustrated

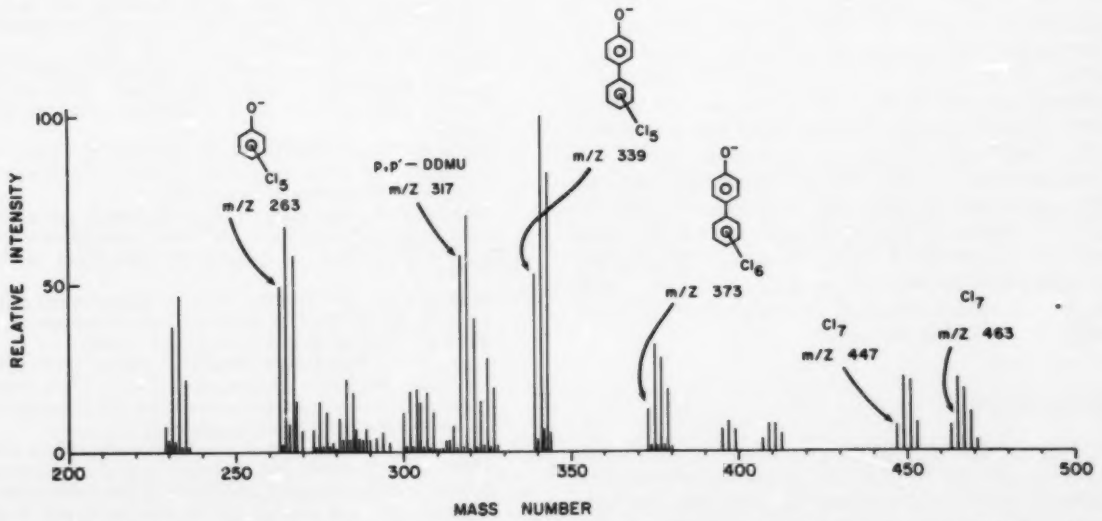

FIGURE 10. Negative chemical ionization mass spectrum of a steam distillate of human adipose tissue. Source conditions equivalent to those in Figure 4.

in Table 3. Table 3 is a dot matrix in which a dot indicates the presence of a given ion in an extract of a seminal fluid sample. Sperm density in the seminal fluid samples increases to the right in Table 3, and ionic clusters observed in the NCI mass spectra increase in mass going down the table. The columns labeled B-1, B-9, and B-11 are procedural blanks. Ions corresponding to hexachlorobiphenyl, heptachlorobiphenyl, and m/z = 463 appeared in the blanks; however, the intensities of these ions were at least a factor of 10 below the intensities normally observed in the seminal fluid samples. It is obvious that we will have to examine a large number of samples before we can make significant statements concerning low sperm densities and the occurrence of individual contaminants or groups of contaminants in the samples.

Screening of Human Tissues For Contamination

We have been applying NCI screening, after a steam distillation cleanup, to human tissues that have been obtained in Medical Examiner autopsy cases. In this screening program we are attempting to establish base line data for human contamination which should be directly complementary to the data that are currently being collected in the EPA human monitoring program. Figure 10 illustrates the NCI mass spectrum of a steam distillate of human adipose tissue. The bulk of the ion current at m/z = 263 in Figure 10 is due to the oxygen exchange reaction occurring on hexachlorobenzene. Oxygen exchange ions appear for the polychlorobiphenyls with five to eight chlorines. The prominent occurrence of nonachlor, chlorodane, and heptachlorepoxide are coincident with findings in the human monitoring program (*31*). DDE appears in the overlapped set of ion clusters at m/z = 351. Table 4 presents a dot matrix summary of the NCI screening results for eight samples of human adipose tissue. We hope that by collection of NCI data on a large number of samples that we will be able to use this technique in epidemiological investigations of environmental health problems.

Summary

The results reported above suggest that negative chemical ionization mass spectrometric screening of environmental samples for contamination with xenobiotic chemicals is passing from the stage of an expensive curiosity to that of a vital research tool in the area of environmental health. NCI screening has revealed the presence of polychlorodibenzofurans and polychlorodibenzodioxins in fish samples obtained from a number of locations in the United States. Remedial action to see that fish from these sources are screened for contamination and do not enter the human food chain may be appropriate. Negative chemical ionization screening of samples of human seminal fluid and human adipose tissue have revealed contamination with a substantial number of toxic substances. The data obtained on these samples is directly complementary to data obtained in the EPA human monitoring program.

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